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Oncogene Activation in Spontaneous and Chemically Induced Rodent Tumors: Implications for Risk Analysis*

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The validity of rodent tumor end points in assessing the potential hazards of chemical exposure to humans is a somewhat controversial but very important issue since most chemicals are classified as potentially hazardous to humans on the basis of long-term carcinogenesis studies in rodents. The ability to distinguish between genotoxic, cytotoxic, or receptor-mediated promotion effects of chemical treatment would aid in the interpretation of rodent carcinogenesis data. Activated oncogenes in spontaneously occurring and chemically induced rodent tumors were examined and compared as one approach to determine the mechanism by which chemical treatment caused an increased incidence of rodent tumors. Different patterns of activated oncogenes were found not only in spontaneous versus chemically induced mouse liver tumors but also in a variety of spontaneous rat tumors versus chemically induced rat lung tumors. In the absence of cytotoxic effects, it could be argued that the chemicals in question activated protooncogenes by a direct genotoxic mechanism. These results provided a basis for the analysis of activated oncogenes in spontaneous and chemically induced rodent tumors to provide information at a molecular level to aid in the extrapolation of rodent carcinogenesis data to human risk assessment.

Introduction

Several approaches have been used to identify environmental agents posing significant carcinogenic hazards to man. These include *in vitro* assays for mutagenesis and *in vivo* tumorigenicity assays. However, most chemicals are classified as potentially hazardous to humans on the basis of long-term carcinogenesis studies in rodents. While these rodent carcinogenesis studies are designed to mimic the route of human exposure in the environment or workplace, the dose of a given chemical is usually higher than that which actually occurs in human exposure. The extrapolation of rodent carcinogenic data to human risk is therefore complicated by the higher doses of chemicals that are employed in the rodent bioassays, especially in the absence of information regarding the mechanism(s) of tumor induction. Another complicating factor in the interpretation of rodent carcinogenic data is the appearance of species- and strain-specific spontaneously occurring tumors in rodents that are used in the bioassays.

Increasing evidence suggests that a small set of cellular genes appear to be targets for genetic alterations that contribute to the neoplastic transformation of cells. These genes, termed protooncogenes, were initially discovered as the transduced oncogenes of acute transforming retroviruses (*1*). Recent studies have established that protooncogenes can also be activated as oncogenes by mechanisms independent of retroviral involvement. These mechanisms include point mutations or gross DNA rearrangements such as translocations or gene amplifications (*1,2*). Protooncogenes appear to play a crucial role in normal cellular growth and/or differentiation since they are highly conserved in nature, being detected in species as divergent as yeast, Drosophila, and humans.

The activation of protooncogenes in spontaneous and chemically induced tumors has been studied in great detail during the past several years. The number of protooncogenes that must be activated in the mul-

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Table 1. Activated oncogenes in liver tumors of the B6C3F1 mouse.

Treatment	Transfection frequency tumors positive/tumors tested	Oncogenes detected				
		Ha-*ras*				
		Codon 61 mutation	Not codon 61 mutation	Ki-*ras*	*raf*	Unknown
Vehicle	17/27	15	—	—	1	1
Chemical	26/45	11	8	3	1	3

tistep process of carcinogenesis is unclear at present although the concerted expression of at least two oncogenes, *ras* and *myc*, are needed for the transformation of a primary cell *in vitro* (*3*). Also, in addition to the activation of protooncogenes, the loss of specific regulatory functions such as tumor suppressor genes may be a distinct step in neoplastic transformation (*4*).

Investigations in rodent models for chemical carcinogenesis imply that oncogenes are activated by carcinogen treatment and that this activation process is an early event in tumor induction (*5–7*). These findings suggest that one approach to determine the mechanism(s) by which a chemical causes an increased incidence of tumors in a rodent bioassay is to compare oncogene activation in spontaneous and chemically induced tumors. If the activated oncogenes detected in chemically induced tumors were different from those found in spontaneous tumors, then one could argue that the chemical activated the protooncogene by a genotoxic mechanism. Alternatively, the detection of similar activating lesions in oncogenes of spontaneous and chemically induced tumors could imply that the chemical was increasing the spontaneous tumor incidence by a mechanism such as cytotoxicity or receptor-medicated promotion.

This paper will discuss the detection of activated oncogenes in spontaneous and chemically induced tumors of the B6C3F1 mouse and the Fischer 344/N rat, two species used extensively in National Toxicology Program/National Cancer Institute (NTP/NCI) carcinogenesis studies. The implications of activated oncogenes in these rodent tumors will also be discussed in terms of the extrapolation of rodent carcinogenic data to human risk assessment.

Activated Oncogenes in Liver Tumors of the B6C3F1 Mouse

DNA transfection analysis of a series of spontaneous (*8,9*) and chemically induced (*10*) liver tumors showed oncogene activation in both benign and malignant tumors. Southern blot analysis indicated a high incidence of Ha-*ras* gene activation in both the spontaneous (*9*) and chemically induced (*10*) liver tumors. Activated Ki-*ras* genes were detected in the chemically induced liver tumors but not in the spontaneous liver tumors (*9,10*). Also, *raf* oncogenes and non-*ras* oncogenes were detected in both the spontaneous and chemically induced liver tumors (*9,10*).

Oligonucleotide analysis indicated that all of the Ha-*ras* oncogenes detected in spontaneous liver tumors were activated by point mutations in codon 61 whereas the Ha-*ras* oncogenes detected in the chemically induced liver tumors were activated by point mutation at a number of different codons (*10*). The detection and analysis of activated oncogenes in mouse liver tumors is summarized in Table 1.

Activated Oncogenes in Tumors of the Fischer 344/N Rat

In contrast to the results obtained with the spontaneous mouse liver tumors, oncogenes detectable by transfection analysis were absent in 9 malignant and 20 benign spontaneous nonliver tumors of the Fischer 344/N rat. These tumors included 8 mammary adenomas or fibroadenomas, 5 testicular interstitial cell adenomas, 4 subcutaneous fibromas or fibroadenomas, 3 mononuclear cell leukemias, 2 fibrosarcomas, and a single adrenal pheochromocytoma, mammary adenocarcinoma, pancreatic acinar adenoma, pancreatic islet cell adenoma, pituitary adenoma, splenic hemangiosarcoma, and prostatic adenocarcinoma (*9*).

DNA transfection analysis of a series of rat lung tumors, generated in a recent long-term carcinogenesis study conducted by NTP, indicated that 18 of 19 tumors contained detectable oncogenes. Southern blot and oligonucleotide analysis indicated that all of the rat lung tumors contained an activated Ki-*ras* gene with a GGT → GAT mutation in codon 12 (*11*). The detection of activated oncogenes in rat tumors is summarized in Table 2.

Interpretation of Rodent Carcinogenesis Data and Its Implications for Human Risk Assessment

The regulation of human exposure to a chemical, based on long-term carcinogenesis studies in rodents, should take into account whether the chemical in question is mutagenic, cytotoxic, or has a receptor-mediated mechanism of promotion. Oncogene analysis of tumors from spontaneous origin and from long-term carcinogenesis studies can be useful in several ways. Investigation of oncogene activation in tumors

Table 2. Activated oncogenes in tumors of the Fischer 344/N rat.

Treatment	Transfection frequency tumors positive/tumors tested	Oncogenes detected, Ki-*ras* Codon 12 mutation
Vehicle	0/29	—
Chemical	18/19	18

will allow an analysis of the mechanism of tumor formation at a molecular level. For instance, the finding of activating mutations in different codons of the Ha-*ras* gene in chemically induced liver tumors versus finding activating mutations in only one codon of the Ha-*ras* gene in spontaneous liver tumors could, in the absence of any cytotoxic effects, be used to argue that the chemical itself activated the Ha-*ras* protooncogene by a genotoxic event. Likewise, different patterns of oncogene activation in spontaneous versus chemically induced rat tumors would also be helpful in the classification of a rodent carcinogen as an initiator, promoter, or cytotoxic chemical. This type of analysis might be of particular importance for compounds which are negative for mutagenicity on short-term tests but which are positive for carcinogenicity in long-term bioassays. Knowledge of the mechanisms by which chemicals induce tumors in long-term rodent bioassays may remove some of the uncertainty of low-dose and species-to-species extrapolation of human risk from rodent carcinogenesis data.

REFERENCES

1. Bishop, J. M. Viral oncogenes. Cell 42: 23–38 (1985).
2. Varmus, H. E. The molecular genetics of cellular oncogenes. Annu. Rev. Genet. 18: 553–612 (1984).
3. Land, H., Parada, L., and Weinberg, R. A. Cellular oncogenes and multistep carcinogenesis. Science 222: 771–778 (1983).
4. Barrett, J. C., Oshimura, M., and Koi, M. Role of oncogenes and tumor suppressant genes in a multistep model of carcinogenesis. In: Symposium on Fundamental Cancer Research, Vol. 38 (F. Becker, Ed.), 1987, in press.
5. Zarbl, H., Sukumar, S., Arthur, A. V., Martin-Zanca, D., and Barbacid, M. Direct mutagenesis of Ha-ras-1 oncogenes by N-nitroso-N-methylurea during initiation of mammary carcinogenesis in rats. Nature (London) 315: 382–385 (1985).
6. Quintanilla, M., Brown, K., Ramsden, M., and Balmain, A. Carcinogen-specific mutation and amplification of Ha-ras during mouse skin carcinogenesis. Nature (London) 332: 78–80 (1986).
7. Bizub, D., Wood, A. W., and Skalka, A. M. Mutagenesis of the Ha-ras oncogene in mouse skin tumors induced by polycyclic aromatic hydrocarbons. Proc. Natl. Acad. Sci.(U.S.) 83: 6048–6052 (1986).
8. Fox, F. R., and Watanabe, P. G. Detection of a cellular oncogene in spontaneous liver tumors of B6C3F1 mice. Science 228: 596–597 (1985).
9. Reynolds, S. H., Stowers, S. J., Maronpot, R. R., Anderson, M. W., and Aaronson, S. A. Detection and identification of activated oncogenes in spontaneously occurring benign and malignant hepatocellular tumors of the B6C3F1 mouse. Proc. Natl. Acad. Sci. (U.S.) 83: 33–37 (1986).
10. Reynolds, S. H., Stowers, S. J., Patterson, R. M., Maronpot, R. R., Aaronson, S. A., and Anderson, M. W. Activated oncogenes in B6C3F1 mouse liver tumors: Implications for risk assessment. Science 237: 1309–1316 (1987).
11. Stowers, S. J., Glover, P. L., Reynolds, S. H., Boone, L. R., Maronpot, R. R., and Anderson, M. W. Activation of the K-ras protooncogene in lung tumors from rats and mice chronically exposed to tetranitromethane. Cancer Res. 47: 3212–3219 (1987).